

Note

Synthesis and Photocleaving DNA Activity of 4'-Chlorobenzene-sulfonyl Chloride-pyrrolicarboxamide Hybrid Compound

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A 4'-chlorobenzenesulfonyl chloride-pyrrolicarboxamide hybrid compound was synthesized and its DNA cleaving activity was investigated using a pBluescript SK DNA under UV irradiation. This compound showed potent DNA cleaving activity.

Keywords photoluclease, DNA cleaving, polyamide

Introduction

Synthetic photoinduced DNA cleavage agents are one of the focuses of chemistry and biology. The appealing feature of photoinduced cleavage is that the agents are inert without irradiation.¹⁻³ The 4'-chloroaromatic derivatives belong to a class of DNA cleavage agents which can initiate the DNA cleavage under light irradiation via production of carbon-centered radical.⁴ Distamycin, an oligopeptide containing three *N*-methylpyrrole rings, which is a naturally occurring antiviral antibiotics, is able to bind to the DNA minor groove preferentially to 5'-AAATT-3' sequence.⁵ The photoinduced DNA cleavage agents are very useful in artificial restriction enzymes, inhibition of gene expression and development of gene-selective drugs in anticancer and antiviral chemotherapy. In this research, a conjugate of 4'-chlorobenzenesulfonyl chloride and distamycin analogue was designed and synthesized (Fig. 1), and its strong DNA cleaving activity under irradiation of UV was observed.

Results and discussion

The synthesis of compound **1** requires two key inter-

mediates, ethyl β -[1-methyl-4-(1-methyl-4-(1-methylpyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido]alaninate [PyPyPy β OEt (**2**)] and 3-(4'-chlorophenylsulfonylamino)-3'-amino-*N*-methyl-di-propylamine (**3**). The intermediate **2** had been prepared^{6,7} successfully through the coupling of 1-methyl-4-(1-methylpyrrole-2-carboxamido)pyrrole-2-carboxylic acid (PyPy-COOH) and ethyl β -[1-methyl-4-aminopyrrole-2-carboxamido]alaninate (H₂NPY β OEt) using DCC/HOBT coupling reaction, followed by saponification of ethyl ester.⁸ On the other hand, **3** was prepared by adding 4'-chlorobenzenesulfonyl chloride to 3,3'-diamino-*N*-methyl-di-propylamine in presence of Et₃N. In the final step, the two intermediates were coupled in the presence of DCC/HOBT in dry DMF, and subsequent purification using column chromatography (0%—2.5% aq. NH₃ in CH₃OH) gave the conjugate **1** (Scheme 1).

The complex of pBluescript SK DNA and **1** was irradiated under UV light (365 nm, 40 W) for 40 min, the DNA cleaving activity of the conjugate **1** was assessed by an electrophoresis method to monitor the conversion of circular supercoiled DNA (form I) into circular nicked DNA (form II). The result of the electrophoresis was shown in Figs. 2 and 3.

From control experiment (Lanes 1 to 3) no obvious DNA cleavage was observed, demonstrating the requirement of irradiation in the DNA cleavage mediated by 4'-chlorophenylsulfonyl chloride-pyrrolicarboxamide conjugate **1**. The concentration of conjugate **1** was ranged in 5—200 $\mu\text{mol} \cdot \text{L}^{-1}$ for the comparison of their DNA cleaving

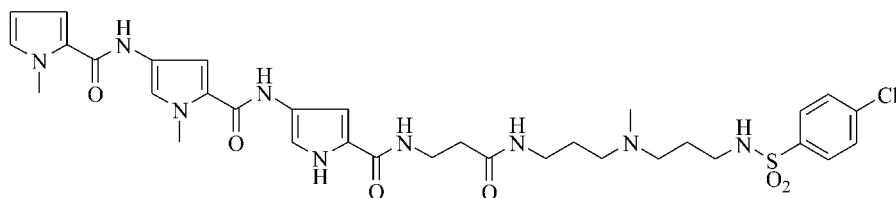
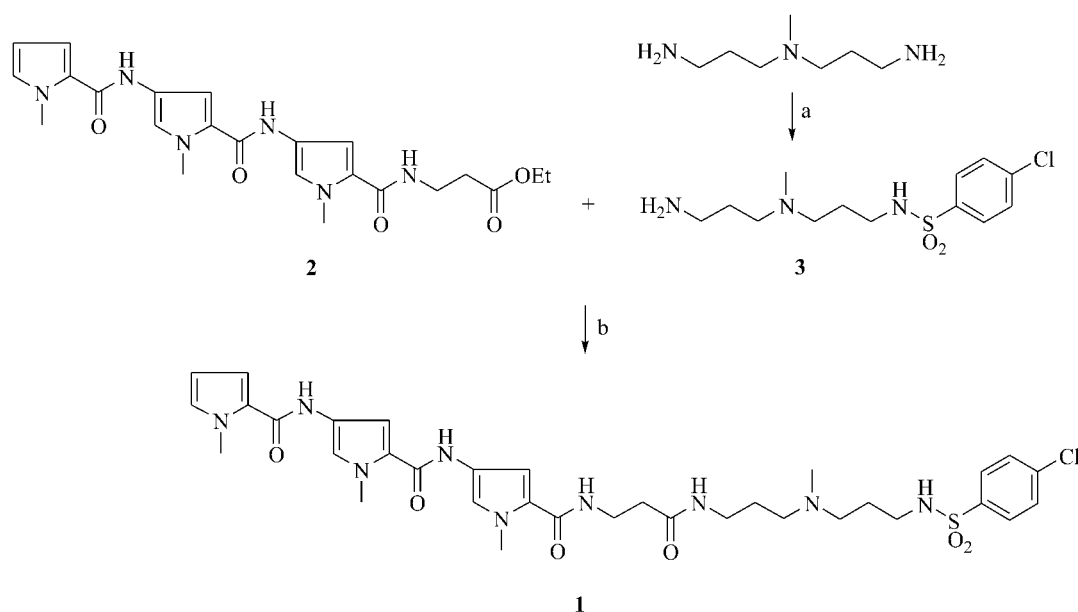


Fig. 1 Structure of 4'-chlorobenzenesulfonyl chloride-pyrrolicarboxamide hybrid compound **1**.

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Scheme 1 Synthesis of conjugate **1**. (a) 4'-Chlorophenylsulfonyl chloride, Et_3N , THF; (b) NaOH, EtOH/ H_2O , $6 \text{ mol} \cdot \text{L}^{-1}$ HCl; DCC/HOBT, DMF.



Fig. 2 Light-induced cleavage of supercoiled DNA (pBluescript SK) by **1**. The supercoiled DNA (pBluescript SK) runs at position I, the nicked DNA at position II. Lane 1, DNA ($15 \mu\text{mol} \cdot \text{L}^{-1}$) alone; Lane 2, DNA alone, irradiated; Lane 3, DNA + **1**, no irradiation; Lane 4—8, DNA + **1** ($200, 150, 100, 50, 5 \mu\text{mol} \cdot \text{L}^{-1}$, respectively), irradiated.

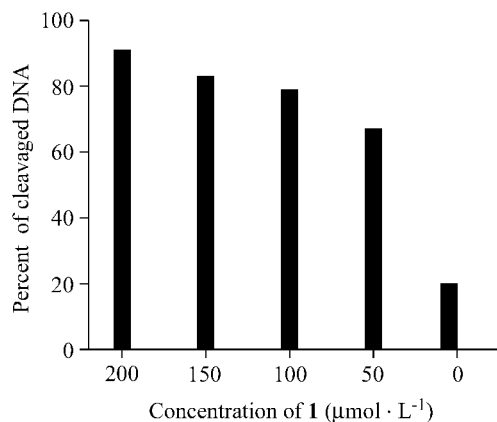


Fig. 3 Quantitative analysis of the DNA cleaving ability of **1**.

activity (Figs. 2 and 3). The photocleaving activities of the conjugate **1** depended on its concentration, *i. e.* with the increase of the concentration of the conjugate **1**, the efficiency of DNA cleaving increased. The amounts of the supercoiled DNA (form I) and the nicked DNA (form II) observed indicated that the conjugate **1** was effective even at

the concentration of $5 \mu\text{mol} \cdot \text{L}^{-1}$. In presence of the conjugate **1** at $200 \mu\text{mol} \cdot \text{L}^{-1}$, 91% conversion of the supercoiled DNA to the nicked DNA was achieved.

In conclusion, 4'-chlorobenzene-sulfonyl chloride-pyrrololecarboxamide conjugate **1** was synthesized successfully and it showed remarkable DNA cleaving activity.

Experimental

Synthesis of 3-(4'-chlorobenzene-sulfonylamino)-3'-amino-N-methyl-dipropylamine (**3**)

To a solution of **3**, 3'-diamino-N-methyl-dipropylamine (0.08 g, 0.55 mmol) and Et_3N (0.08 mL, 0.55 mmol) in THF (7 mL) was added 4'-chlorobenzene-sulfonyl chloride (0.12 g, 0.55 mmol). The reaction mixture was stirred for 4 h. The solution was evaporated *in vacuo*. The residue was purified by column chromatography (0%—2.5% aq. NH_3 in CH_3OH) to afford the slight yellow liquid **3** (0.09 g, 50% yield). $^1\text{H NMR}$ (CDCl_3 , 200 MHz) δ : 1.58—1.70 (m, 4H), 2.16 (s, 3H), 2.38—2.45 (m, 4H), 2.84 (t, $J = 6.6 \text{ Hz}$, 3H), 3.03 (t, $J = 5.8 \text{ Hz}$, 2H), 4.61 (br, 2H), 7.44—7.51 (m, 2H), 7.79—7.86 (m, 2H); IR (KBr) ν : 3088, 2948, 2852, 2804, 1653, 1587, 1476, 1328, 1161, 1094, 755, 619 cm^{-1} . FAB-MS calcd for $\text{C}_{13}\text{H}_{23}\text{N}_3\text{SO}_2\text{Cl}$ (MH^+) 320, found 320.

Synthesis of ethyl β -{1-methyl-4-[1-methyl-4-(1-methyl-pyrrole-2-carboxamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido} alaninate-4'-chlorobenzene-sulfonyl chloride conjugate (**1**)

After $\text{PyPyPy}\beta\text{OEt}^{6,7}$ was saponified with NaOH and neutralized with hydrochloric acid,⁸ to a solution of

PyPyPy β COOH (0.21 g, 0.47 mmol) in 4 mL of DMF was added HOBT (0.07 g, 0.50 mmol), followed by DCC (0.11 g, 0.50 mmol). The reaction mixture was stirred for 12 h. Then, 0.15 g (0.47 mmol) of **3** was added and stirred for 12 h. The solution was filtered and evaporated to remove DCU and DMF *in vacuo*, correctively. The residue was purified by column chromatography (0%—2.5% aq. NH₃ in CH₃OH), slight yellow solid **1** was obtained (0.29 g, 83% yield; purity 98%, HPLC determination). ¹H NMR (CDCl₃, 200 MHz) δ : 1.55—1.67 (m, 4H), 2.07 (s, 3H), 2.28—2.35 (m, 4H), 2.51—2.55 (m, 2H), 2.95—3.00 (m, 2H), 3.24 (d, *J* = 5.8 Hz, 2H), 3.66 (d, *J* = 4.8 Hz, 2H), 3.80 (s, 3H), 3.88 (s, 3H), 3.97 (s, 3H), 6.08—6.12 (m, 1H), 6.67 (s, 1H), 6.74 (s, 2H), 6.81 (s, 1H), 6.90—6.91 (m, 2H), 7.06 (t, *J* = 3.4 Hz, 1H), 7.23 (s, 1H), 7.39 (s, 1H), 7.45 (d, *J* = 8.5 Hz, 2H), 7.83 (d, *J* = 8.5 Hz, 2H), 8.53 (s, 1H), 8.69 (s, 1H); IR (KBr) ν : 3445, 1645, 1417, 1317, 1160, 1094, 753 cm⁻¹. HRMS calcd for C₃₄H₄₅N₉SO₆Cl (MH⁺) 742.2899, found 742.2898.

DNA photocleavage experiment

A pBluescript SK supercoiled DNA (15 μ mol · L⁻¹) was incubated with **1** for 2 min in DMSO:Tris = 1:9 buffer

(20 mmol · L⁻¹, pH = 7.5), and then was irradiated for 40 min by light from UV lamp (365 nm, 40 W). After photolysis was complete, the sample was analyzed by gel electrophoresis (0.7% agarose gel, ethidium bromide staining) and the amount of the supercoiled DNA (form I) to the nicked DNA (form II) was quantitated by densitometry.

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